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Seasonal proteome changes of nasal mucus reflect perennial inflammatory response and reduced defence mechanisms and plasticity in allergic rhinitis

Peter Valentin Tomazic [a,*,1], Ruth Birner-Gruenberger [b,c,d,**,1], Anita Leitner [a], Stefan Spoerk [b,d,e], Doris Lang-Loidolt [a,2]

[a] Medical University of Graz, ENT—University Hospital, Graz, Austria
[b] Medical University of Graz, Institute of Pathology, Research Unit Functional Proteomics and Metabolic Pathways, Graz, Austria
[c] Austrian Center of Industrial Biotechnology, Graz, Austria
[d] Omics Center Graz, BioTechMed-Graz, Graz, Austria
[e] Medical University of Graz, Center of Medical Research, Mass Spectrometry Core Facility, Graz, Austria

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ABSTRACT

Introduction: Nasal mucus and its proteins are a defence against allergens. We sought to investigate dynamic proteome changes in allergic rhinitis upon environmental allergen provocation.
Methods: Nasal mucus was collected in and out of pollen season from allergic rhinitis patients (N = 10) and healthy controls (N = 12). Liquid chromatography–tandem mass spectrometry was performed. Proteins were identified by SwissProt database search and quantified from normalized areas under curve of precursor ion chromatograms. Gene enrichment analysis was performed with Cytoscape/BINGO software.
Results: In total 430 different proteins were detected in both groups, 203 (47.2%) were newly identified. In allergics CLU and IGKC were significantly more abundant in season (2.2 and 2.1-fold respectively). GSTP1 (0.5-fold), ELANE (0.4-fold), HIST1H2BK (0.3-fold), S100A8 (0.2-fold), S100A12 (0.2-fold) and ARHGDIB (0.1-fold) were significantly less abundant in season. In healthy controls UBC, TUBA1B, HBB and FABP5 were only present in season. Ig kappa chain V-I region DEE (5.3-fold), CLU (5.0-fold), TXN (4.3-fold), MSMB (3.2-fold) and Ig heavy chain V-III region BRO (2.7-fold) were significantly more abundant in season. MUC5B (0.5-fold), SLPI (0.2-fold) and S100P (0.2-fold) were significantly less abundant in season.
Conclusion: Contrary to their symptoms allergic rhinitis patients show perennial inflammatory response lacking adequate reaction to allergens in season.
Biological significance: Many studies dealing with allergic rhinitis are focused on the nasal epithelium. This is the first study to analyse the nasal mucus as primary defence barrier on a proteomic level in and out of pollen season and contrary to the leading opinion shows that allergic patients show a perennial inflammatory response with reduced reaction to allergens whereas healthy controls react on proteome basis towards enhanced defence in season despite lacking allergic sensitization.

1. Introduction

Allergic rhinitis is a global health problem affecting up to 40% of the population in some regions regardless of gender or age [1]. The pathophysiology of allergic rhinitis is well understood with affected subjects being sensitized to otherwise harmless inhaled allergens. Upon contact with the patients' immune system immune cells produce IgE instead of IgG which causes mast cell degranulation and the classical symptoms of nasal congestion and rhinorrhea [1,2]. Nevertheless the process of sensitization is unclear. Environmental and many other factors have been proposed to explain why certain individuals develop allergy and some not [3]. Nasal mucus is the first line defence barrier against allergens and its proteins might play a role in the sensitization process and allergen interaction with the underlying epithelium [4,5].

Abbreviations: AUC [%], mean normalized areas under the curve; CID, collision induced dissociation; DTT, dithiothreitol; Fw-rev, forward-reverse score; IAA, iodoacetamide; ICR, ion cyclotron resonance; LC–MS/MS, liquid chromatography–tandem mass spectrometry; nano-HPLC, nano-flow-high performance liquid chromatography; SD, standard deviation; SEM, standard error of mean; %SPI, Scored Peak Intensity Percent; SPT, skin prick test.

* Correspondence to: P.V. Tomazic, ENT—University Hospital Graz, Medical University of Graz, Auenbruggerplatz 26, 8036 Graz, Austria.
** Correspondence to: R. Birner-Gruenberger, Institute of Pathology, Medical University of Graz, Stiftingtalstrasse 24, 8036 Graz, Austria.
E-mail addresses: peter.tomazic@medunigraz.at (P.V. Tomazic), ruth.birner-gruenberger@medunigraz.at (R. Birner-Gruenberger).
[1] Peter Valentin Tomazic and Ruth Birner-Gruenberger contributed equally to this article.
[2] Doris Lang-Loidolt should be considered as senior author.

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A possible role of nasal mucus could be to block pollen proteases with innate antiproteases and to prevent subsequent epithelial damage and allergen transport through the mucosa [6–9]. Proteins exudated from plasma could have immunomodulatory functions and influence disease severity [10,11]. To understand the nasal mucus proteome proteomic studies are feasible detecting a large range of proteins and facilitating analysis of their origin and function [12].

We recently showed that there are significant differences in nasal mucus proteome between allergic rhinitis patients and healthy controls, and that some proteins might be novel biomarkers and lead to better understanding of the disease [13,14] Moreover, we validated our methodology and confirmed our mass spectrometry results by Western Blotting of selected proteins [13,14]. In these previous studies patients were analyzed at a single time point solely and thus seasonal differences of the proteome could not be assessed. The present study is aimed at resolving seasonal proteome changes in allergic rhinitis patients and healthy controls to reveal whether pollen exposure leads to changes of the nasal mucus proteome in dependence of disease. We therefore collected nasal mucus of allergic rhinitis patients and healthy controls at two distinct time points, i.e. in and out of pollen season. The obtained paired dynamic proteome profiles were examined with respect to how either group reacts to natural pollen exposure on a proteome level. Our results further stratify and identify novel key proteins playing a role in allergic rhinitis.

2. Material and methods

2.1. Patients

Sixty-eight individuals were enrolled in the study, with a drop out number of 46, because they did not appear to the follow-up controls or did not meet the inclusion criteria at the follow-up visit anymore, like usage of topical and/or systemic corticosteroids, antihistamines or any other immunomodulatory drugs. The remaining twenty-two individuals (7 males, 15 females) with a mean age of 33 years (SD: 9.7 years) were included in the study group comprising 10 (45%) allergic rhinitis patients and 12 (55%) healthy controls. Allergy status was verified by skin prick tests (SPT, Allergopharma GmbH & Co. KG, Reinbek, Germany) and specific IgE (ImmunoCAP, Thermo Fisher Scientific Inc., Vienna, Austria) in all patients and controls. Patients sensitized to house dust mite or animals solely were excluded to avoid bias due to small sample size (Table 1 and supplementary Table E1). Thus, only patients sensitized to pollen and also showing symptoms during the pollen season were considered for evaluation. Patients with acute and/or chronic sinusitis as defined by the EPOS [15] guidelines were also excluded, as were patients with malignant tumors and any infectious or cardiopulmonary disease, or who had been treated with systemic or topical drugs including antihistamines, corticosteroids, antibiotics, antifungals or any other immunomodulatory drugs in the four weeks prior to the study. The same exclusion criteria applied to the controls, who were healthy volunteers recruited from the hospital staff. Informed consent was obtained from all participants (allergics and controls) before enrolment. The study was approved by the institutional review board of the Medical University of Graz.

Table 1
Summarized epidemiologic data with distribution of gender, age, group as well as skin prick test (SPT) for clinically relevant allergens, symptoms present and total IgE.

Parameter	Allergic rhinitis	Healthy controls
Number of patients	10	12
Demographics		
Mean age, years (SD)	30.4 (8.4)	36.3 (10.1)
Women, %	75%	40%
Clinically relevant positive SPT		
Alder/Hasel/Birchpollen	8	0
Grasspollenmix	9	0
Ragweedpollen	3	0
Symptoms during pollenseason		
Allergic rhinitis	10	0
Allergic rhinitis and asthma	0	0
Total IgE (kU/l), (SEM)	136.1 (122.1)	42 (37.5)

SD: standard deviation.
SPT: skin prick test.
SEM: standard error of mean.

2.2. Sample collection

In pollen season (with clinical symptoms present in allergic rhinitis patients) and out of pollen season (without clinical symptoms in allergic rhinitis patients) nasal mucus was collected with a special suction device (Sinus Secretion Collector, Medtronic Xomed Inc., Jacksonville, Florida, USA). Healthy controls' samples were collected on the same day as allergic rhinitis patients' samples. Without previous interventions (decongestants, local anesthetics) untreated mucus was obtained under endoscopic control from the nasal cavity and middle meatus with meticulous care taken not to touch the mucosa. The mucus volume obtained was equal in both groups. Then, mucus was deep-frozen at −92 °C before processing for LC–MS/MS mass spectrometry.

For sample preparation and mass spectrometric analysis please see methods supplement in the online repository. Proteomic experiments were performed according to MIAPE (minimum information about a proteomic experiment) [14,16].

2.3. Data analysis

The LC–MS/MS data were analyzed by searching the human SwissProt public database (downloaded on March 10th 2012) with Proteome Discoverer 1.4 (Thermo Scientific) and Mascot 2.2 (Matrix Science, London, UK). Detailed settings: Enzyme: trypsin, max. missed cleavage sites: 2, N-terminus: hydrogen, C-terminus: free acid, carbamidomethylation on lysine as fixed modification, oxidized methionine as variable modification, maximum precursor charge 3; precursor mass tolerance +/−0.05 Da, product mass tolerance +/−0.7 Da; acceptance parameters were 2 or more identified distinct peptides after automatic validation (decoy search, FDR < 5%). Identified proteins were annotated using data from Uniprot (www.uniprot.org). Areas under the curve (AUCs) (i.e. mean areas of extracted ion chromatograms of the individual peptides matched to a protein) normalized on the total AUC of all proteins in each sample were used to compare relative protein abundances of the same protein between groups [17]. Data are reported as means and standard errors of mean (SEM). Statistical analysis by Mann Whitney U test for seasonal or group differences and multivariate analysis for seasonal and group differences were performed with SPSS 18.0 software (Chicago, Illinois, USA). A p-value of <0.05 was considered significant.

Enrichment analysis was performed with BINGO 2.44 [18] in Cytoscape 2.81 software (www.cytoscape.org) [18]. For statistical analysis of enrichment data created with BINGO/Cytoscape hypergeometric tests were performed and corrected by Benjamini & Hochberg False Discovery Rate (FDR) correction at a significance level of 0.05.

3. Results

The mean total protein concentration over all samples was 0.58 mg/ml (SD: 0.66). Allergic rhinitis patients (AR) had a higher mean protein concentration (0.78 mg/ml (SD: 0.87)) than healthy controls (HC) (0.42 mg/ml (SD: 0.35)). However, this difference was not significant ($p = 0.377$). Overall 430 different proteins were detected in both groups, while 327 were detected in AR and 366 in HC respectively. Of these 203 proteins (47.2%) were newly identified as nasal mucus proteins (supplementary Table E2). The most abundant proteins were Serum albumin (ALB), Lysozyme C (LYZ), Ig alpha-1 chain C region (IGHA1) and Ig alpha-2 chain C region (IGHA2) independent of disease

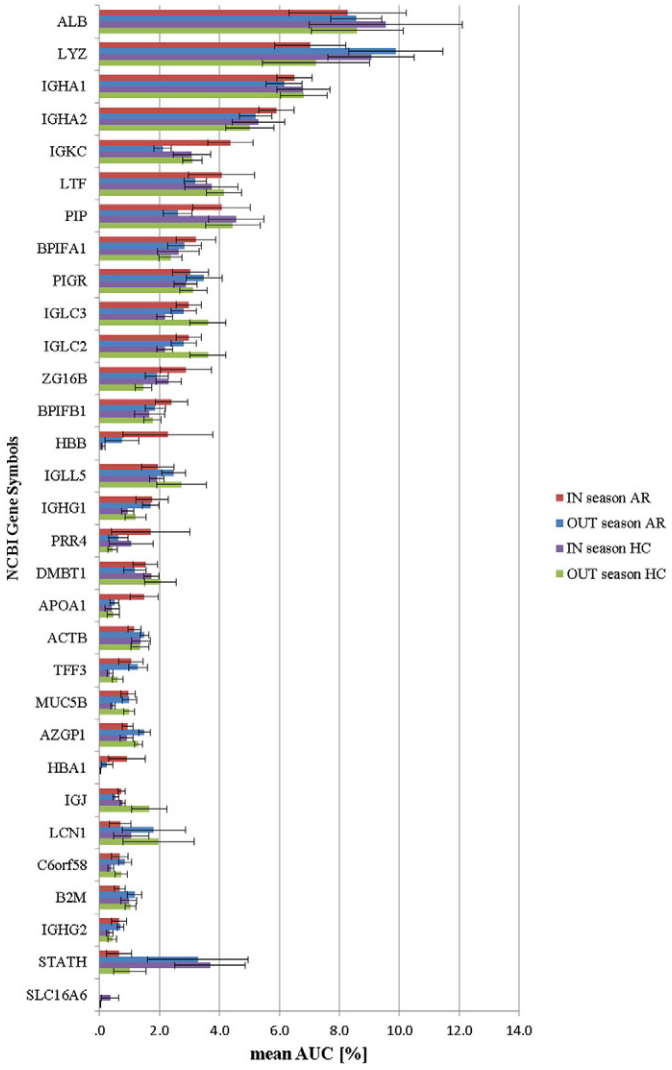

Fig. 1. Differences in mean normalized areas under the curve between allergic rhinitis patients (AR) and healthy controls (HC) IN and OUT of season for the 30 most abundant proteins.

and season. Statherin (STATH) significantly showed the highest diametric abundance change with respect to group and seasonal differences (Fig. 1).

In AR patients 280 proteins were detected in season and 259 out of season. Of these 211 proteins were identified in and out of pollen season, 69 solely in season (with 36 exclusively identified in AR) and 48 solely out of season (with 18 exclusively in AR). In HC 328 proteins were detected in season, and 242 out of season. Of these 204 proteins were found in and out of season, 124 solely in season (with 65 exclusively in HC) and 38 solely out of season (with 22 exclusively in HC). The difference in abundance between the groups for proteins present only either in or out of season was highly significant ($p = 0.004$) (Fig. 2).

3.1. Seasonal differences in allergic rhinitis patients

In AR patients eight proteins showed significantly different abundances in season as compared to out of pollen season. These were Clusterin (CLU), Ig kappa chain C region (IGKC), Glutathione S-transferase P (GSTP1), Neutrophil elastase (ELANE), Histone H2B type 1-K (HIST1H2BK), Protein S100-A8 (S100A8), Protein S100-A12 (S100A12) and Rho GDP-dissociation inhibitor 2 (ARHGDIB). Of these CLU and IGKC were significantly more abundant in season (2.2 and

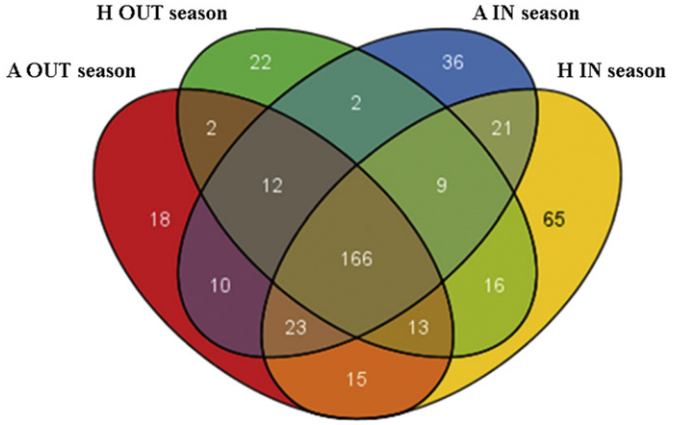

Fig. 2. Venn diagram of protein distribution (number of proteins) between groups and seasons. A: allergics, H: healthy controls.

2.1-fold respectively). GSTP1 (0.5-fold), ELANE (0.4-fold), HIST1H2BK (0.3-fold), S100A8 (0.2-fold), S100A12 (0.2-fold) and ARHGDIB (0.1-fold) were significantly less abundant in season (Table 2).

3.2. Seasonal differences in healthy controls

In HC 12 proteins showed significantly different abundances between the seasons. These were Polyubiquitin-C (UBC), Tubulin alpha-1B (TUBA1B), Hemoglobin beta (HBB), fatty acid-binding protein, epidermal (FABP5), Ig kappa chain V-I region DEE (N/A), Clusterin (CLU), Thioredoxin (TXN), Beta-microseminoprotein (MSMB), Ig heavy chain V-III region BRO, Mucin-5B (MUC5B), Antileukoproteinase (SLPI) and Protein S100-P (S100P). Of these UBC, TUBA1B, HBB and FABP5 were only present in pollen season. Ig kappa chain V-I region DEE (5.3-fold), CLU (5.0-fold), TXN (4.3-fold), MSMB (3.2-fold) and Ig heavy chain V-III region BRO (2.7-fold) were significantly more abundant in season as compared to out of pollen season. MUC5B (0.5-fold), SLPI (0.2-fold) and S100P (0.2-fold) were significantly less abundant in season (Table 2).

3.3. In season differences between allergic rhinitis patients and healthy controls

In pollen season 10 proteins were significantly more abundant in allergic rhinitis patients than in healthy controls. These were Complement C4-B (C4B), Alpha-1-acid glycoprotein 2 (ORM2), and Phospholipid transfer protein (PLTP), which were not detected in HC at all; as well as Alpha-2-macroglobulin (A2M, 13.2-fold), Apolipoprotein A-II (APOA2, 9.4-fold), Vitamin D-binding protein (GC, 4.6-fold), Complement C3 (C3, 3.6-fold), Apolipoprotein A-I (APOA1, 3.6-fold), BPI fold-containing family B member 2 (BPIFB2, 2.9-fold) and Clusterin (CLU, 2.6-fold) (Table 3).

3.4. Seasonal differences in biological processes of allergic rhinitis patients' and healthy controls' proteome

In total 22 biological processes (BPs) were enriched in the overall nasal mucus proteome of the whole study group compared to the total human proteome. In allergic rhinitis patients nine BPs were up-regulated in season while 14 were up-regulated out of season (Fig. 3ab, supplementary Table E3). In healthy controls 21 BPs were up-regulated in season and 4 up-regulated out of pollen season respectively (Fig. 4ab, supplementary Table E3). The following BPs were exclusively found in healthy controls: anatomical structure morphogenesis, transport, cell differentiation, death as well as cell death. Vice versa, the only BP not found in HC regardless of season of the processes found to be enriched in allergic rhinitis patients was behavior.

Table 2
Significant proteins for seasonal differences in allergic rhinitis patients and healthy controls with mean normalized areas under the curve (AUC [%]), SEM = standard error of mean. Ratio of mean (AUC [%]) of IN vs. OUT of season differences is presented. P. in "N" means presence of protein in number of patients/total number of probands, Acc. no. means accession number obtained from SwissProt database. A p-value of <0.05 was considered significant and was obtained by Mann Whitney U-Test.

				Timepoint									
				IN season			OUT season				Total		
Group	Acc. no.	NCBI gene names (primary)	p-Value	Mean AUC [%]	SEM AUC [%]	P. in "N"	Mean AUC [%]	SEM AUC [%]	P. in "N"	Ratio IN vs. OUT season	Mean AUC [%]	SEM AUC [%]	P. in "N"
Allergic	P10909	CLU	0.027	0.22	0.03	10/10	0.10	0.04	5/10	2.2	0.16	0.03	15/20
	P01834	IGKC	0.008	4.36	0.76	10/10	2.11	0.29	10/10	2.1	3.24	0.47	20/20
	P09211	GSTP1	0.037	0.17	0.07	5/10	0.37	0.06	1/10	0.5	0.27	0.05	15/20
	P08246	ELANE	0.039	0.07	0.04	3/10	0.19	0.04	8/10	0.4	0.13	0.03	11/20
	O60814	HIST1H2BK	0.029	0.21	0.08	6/10	0.65	0.21	1/10	0.3	0.43	0.12	16/20
	P05109	S100A8	0.047	0.09	0.03	5/10	0.38	0.17	9/10	0.2	0.24	0.09	14/20
	P80511	S100A12	0.045	0.04	0.03	2/10	0.19	0.06	7/10	0.2	0.12	0.04	9/20
	P52566	ARHGDIB	0.034	0.02	0.02	1/10	0.15	0.04	6/10	0.1	0.09	0.03	7/20
Healthy	P0CG48	UBC	0.014	0.11	0.05	6/12	0.00	0.00	0/12	N/A	0.05	0.03	6/24
	P68363	TUBA1B	0.014	0.18	0.09	6/12	0.00	0.00	0/12	N/A	0.09	0.05	6/24
	P68871	HBB	0.037	0.13	0.05	5/12	0.00	0.00	0/12	N/A	0.06	0.03	5/24
	Q01469	FABP5	0.005	0.07	0.02	7/12	0.00	0.00	0/12	N/A	0.03	0.01	7/24
	P01597	N/A	0.021	0.44	0.13	7/12	0.08	0.06	2/12	5.3	0.26	0.08	9/24
	P10909	CLU	0.029	0.08	0.02	8/12	0.02	0.01	2/12	5.0	0.05	0.01	10/24
	P10599	TXN	0.006	0.14	0.04	10/12	0.03	0.02	3/12	4.3	0.09	0.02	13/24
	P08118	MSMB	0.027	0.16	0.05	7/12	0.05	0.05	1/12	3.2	0.10	0.03	8/24
	P01766	N/A	0.000	0.59	0.08	12/12	0.22	0.06	8/12	2.7	0.40	0.06	20/24
	Q9HC84	MUC5B	0.042	0.45	0.07	11/12	0.98	0.18	12/12	0.5	0.72	0.11	23/24
	P03973	SLPI	0.005	0.58	0.15	10/12	2.44	0.57	11/12	0.2	1.51	0.35	21/24
	P25815	S100P	0.008	0.02	0.02	1/12	0.10	0.03	8/12	0.2	0.06	0.02	9/24

Acc. no. = accession number obtained from UniProt database.
SEM = standard error of mean.
AUC = mean normalized area under the curve.
P. in "N" A or H = presence of protein in number of patients/total number of probands.

4. Discussion

Nasal mucus is the first line defence barrier of the nasal mucosa against various inhaled noxa like allergens. Its proteins harbor specific functions that could play a role in the pathophysiology of allergic rhinitis. We recently demonstrated that, on a proteome level, nasal mucus of allergic rhinitis patients and healthy controls showed significant differences [14]. These mainly addressed immune response, mucus and mucosal homeostasis influencing epithelial permeability, and protease/antiprotease balance. Furthermore potential new biomarkers such as apolipoproteins were identified to be enriched in allergic rhinitis that could play an immunomodulatory role apart from their function in lipid metabolism [13,19–23].

In this study we performed a seasonal analysis of the nasal mucus proteome in allergic rhinitis to obtain information about its changes caused by natural pollen exposure. Therefore, mucus from all participants was obtained at two time points, in and out of pollen season. Pollen exposure dependent nasal mucus proteome changes were assessed both in allergic rhinitis patients and healthy controls. Among the most abundant proteins in nasal mucus changes were unremarkable between the two groups as well as between in and out of pollen season and showed a high overlap with our previous results (Figs. 1 and 2) [14]. Interestingly the nasal mucus of allergic rhinitis patients contained significantly fewer proteins exclusively in season compared to healthy controls (68 vs. 124) resembling a higher diversity of proteins in healthy controls as a reaction to pollen exposure. The numerical change of different proteins exclusively present in as compared to out of season was markedly higher in healthy controls (124 vs. 38) compared to allergic rhinitis patients (68 vs. 48). These data suggest a higher plasticity of the healthy compared to the allergic mucus proteome.

Table 3
Significantly different proteins in season between allergic rhinitis patients and healthy controls with mean normalized areas under the curve (AUC [%]), SEM = standard error of mean. Ratio of mean (AUC[%]) of IN vs. OUT of season differences is presented. P. in "N" means presence of protein in number of patients/total number of probands, Acc. no. means accession number obtained from SwissProt database. A p-value of <0.05 was considered significant and was obtained by Mann Whitney U-Test.

Acc. no.	NCBI gene names (primary)	p-Value	Mean AUC [%] allergic	SEM AUC [%]	P. in "N"	Mean AUC [%] healthy	Sem AUC [%]	P. in "N"	Ratio A vs. H IN season
P55058	PLTP	0.029	0.1	.1	4/10	0.0	0.0	0/12	N/A
P19652	ORM2	0.010	0.1	.0	5/10	0.0	0.0	0/12	N/A
P0C0L5	C4B	0.010	0.1	.0	5/10	0.0	0.0	0/12	N/A
P01023	A2M	0.002	0.2	.1	7/10	0.0	.0	2/12	13.2
P02652	APOA2	0.001	0.5	.2	9/10	0.1	.0	3/12	9.4
P02774	GC	0.034	0.2	.1	7/10	0.1	.0	6/12	4.6
P01024	C3	0.043	0.3	.1	7/10	0.1	.0	6/12	3.6
P02647	APOA1	0.011	1.5	.5	10/10	0.4	.2	10/12	3.6
Q8N4F0	BPIFB2	0.017	0.4	.1	10/10	0.1	.0	8/12	2.9
P10909	CLU	0.002	0.2	.0	10/10	0.1	.0	8/12	2.6

A: allergic rhinitis patients, H: healthy controls.
Acc. no.: accession number obtained from UniProt database.
AUC: mean normalized area under the curve.
SEM: standard error of mean.
P. in "N" A or H: presence of protein in number of patients/total number of probands.

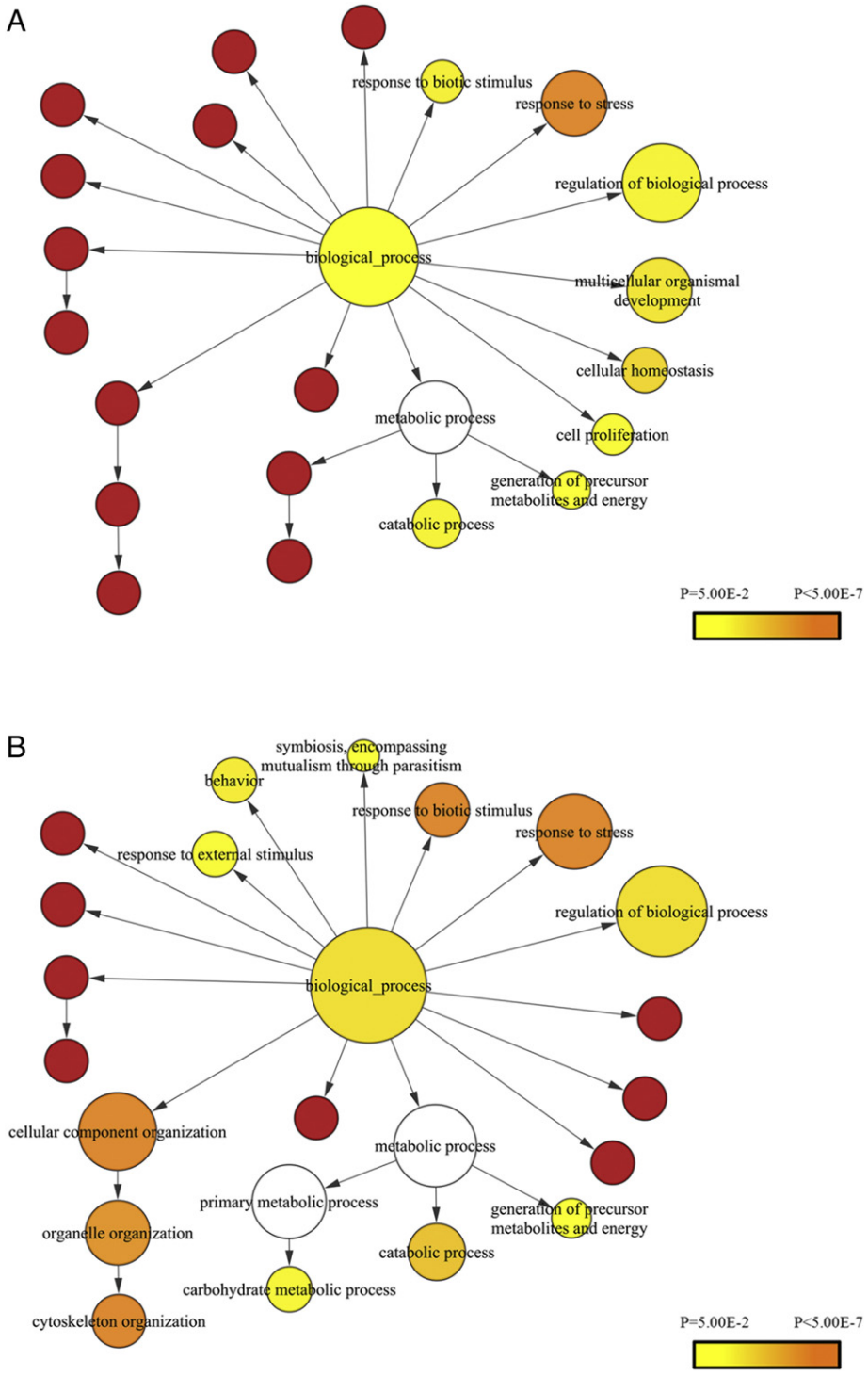

Fig. 3. Enrichment analysis of biological processes, obtained by BINGO software. Significantly enriched biological processes of proteins present in allergic rhinitis patients (N = 17) compared to total human proteome, 9 of these up-regulated in season a) and 14 up-regulated out of season b). Red nodes indicate silenced biological processes. Colour bar in the right lower quadrant indicates level of significance from low (yellow) to high (orange). Statistical analysis was performed with a hypergeometrical test. A p-value of <0.05 was considered significant.

Around 10% of the proteins are ubiquitously expressed or exudated from the plasma. Five percent are produced by the epithelium and goblet cells and around 11% originate from immune cells (see tissue specificity in Table E3). The vast majority of proteins has not been described so far or been allocated to the nasal mucus underlying the diversity of its proteome and potential impact on disease. Correlation of nasal mucus protein expression to epithelial protein expression and plasma protein levels in the future will further facilitate elucidation of the protein origins.

Only Clusterin (CLU) and Ig kappa chain C region (IGKC) were significantly more abundant in allergic rhinitis patients in as compared to out of season; while six proteins were significantly more abundant out of season: Glutathione S-transferase P (GSTP1), Neutrophil elastase (ELANE), Histone H2B type 1-K (HIST1H2BK), Protein S100-A8 (S100A8), Protein

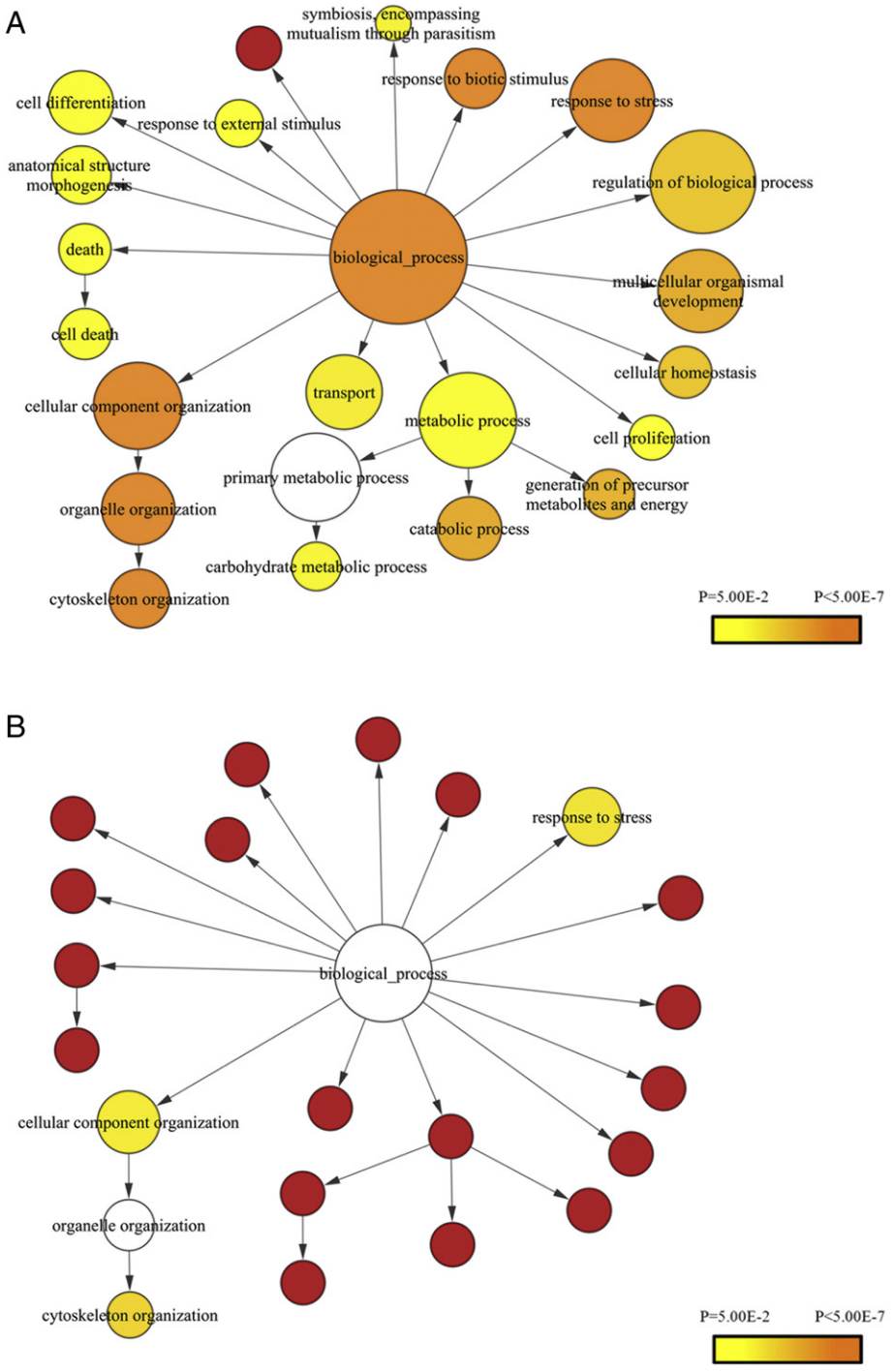

Fig. 4. Enrichment analysis of biological processes, obtained by BINGO software, of significantly enriched biological processes of proteins present in healthy controls (N = 21) compared to total human proteome, 21 of these up-regulated in season a) and 4 up-regulated out of season b). Red nodes indicate silenced biological processes. Colour bar in the right lower quadrant indicates level of significance from low (yellow) to high (orange). Statistical analysis was performed with a hypergeometrical test. A p-value of <0.05 was considered significant.

S100-A12 (S100A12) and Rho GDP-dissociation inhibitor 2 (ARHGDIB). GSTP1 negatively regulates acute inflammatory response and apoptosis [24]. ELANE, S100A8 and S100A12 contrarily have proinflammatory functions promoting neutrophil chemotaxis, chemokine and cytokine production. ELANE furthermore destroys bacteria through its serine protease activity [25–27]. Elevated usually intracellularly found proteins, like histone protein HIST1H2BK and Rho protein regulator ARHGDIB may be a result of tissue damage and/or repair. ARHGDIB negatively regulates cell adhesion and cytoskeleton organization, which can influence the immune response [28,29]. On the other hand, the cytoskeleton protein TUBA1B was found in season exclusively in healthy controls. It is a major constituent of microtubules whose functions are cell organelle positioning, intracellular trafficking, cell polarization and anchoring tight junction proteins giving epithelial cells an apico-basal orientation for enhanced epithelial integrity. In this respect Statherin is another interesting protein showing the most striking diametric abundance changes with respect to seasonal as well as intergroup differences (Fig. 1). It is most abundant in allergic rhinitis patients out of season and in healthy controls in season. It strengthens epithelial integrity through cell organization, motility, polarity and anchoring

tight junctions promoting cell adhesion and thus stabilizes the nasal mucus [30]. This underlines that on a proteome level nasal mucus of allergic rhinitis patients shows an impaired defence during pollen season and that remodeling as well as inflammatory responses are prolonged outside pollen season, contrarily healthy controls show immune response and enhanced defence capacity of the nasal mucus and subsequently the epithelium in season as a consequence to pollen exposure.

Next to TUBA1B healthy controls had 8 other significantly more abundant proteins in their nasal mucus in season compared to out of season: Polyubiquitin-C (UBC), Hemoglobin beta (HBB), FA-binding protein epidermal (FABP5), Ig kappa chain V-I region DEE and Ig heavy chain V-III region BRO, Clusterin (CLU), Thioredoxin (TXN), and Beta-microseminoprotein (MSMB) with the first three and TUBA1B exclusively present in season. UBC when covalently bound to target proteins leads to degradation of the latter via the proteasome suggesting a high degree of protein turnover in healthy controls in season [31,32]. Secreted CLU protects from apoptosis and cytolysis by complement inhibition and promotes cell interaction, membrane protection and tissue repair [33–35]. CLU was also found to be significantly more abundant in season in allergic rhinitis patients suggesting that the CLU dependent tissue protection mechanism is intact in allergic rhinitis. FABP5 is a member of the fatty acid binding protein family which is involved in fatty acid transport and metabolism. It is important for the development of keratinocytes and epidermal appendices like sebaceous glands. FABP5 is responsible for the integrity of the cutaneous barrier, but could also share the same function for the mucosal barrier [36]. Despite mucus was meticulously obtained from the middle meatus it is more likely that FABP5 originates from the nasal vestibule and migrated into the nasal cavity by mucociliary transport.

TXN regulates reactive oxidative metabolism playing an important role in scavenging reactive oxygen species. It was shown to suppress inflammation in an animal asthma model and eosinophil recruitment as well as goblet cell hyperplasia [37–40]. MSMB is known for its presence in prostate and involvement in prostate cancer [41], but was recently shown to be down-regulated in respiratory syncytial virus infection of the nasopharynx [42] and in asthmatic patients [43]. Thus TXN and MSMB may have protective effects for the epithelium in healthy controls during pollen season.

On the other hand, three proteins, namely Mucin-5B (MUC5B), Antileukoproteinase (SLPI) and Protein S100-P (S100P) were significantly more abundant out of season as compared to in season in healthy controls. MUC5B is a major constituent of nasal mucus influencing its viscosity. S100P is involved in the formation of microvilli thus responsible for a normal mucosal cell function and mucociliary clearance. These proteins reflect a normal state of the mucus [5,44–46]. SLPI has antiprotease activity for trypsin, chymotrypsin, elastase, and cathepsin G. Its function is to prevent protease damage to the mucosa [47–51]. SLPI was also significantly more abundant in healthy controls as compared to allergic rhinitis patients in our previous study as well as other antiproteases [13,14]. However, it has been published that proteases from pollen are only sufficiently inhibited by cysteine antiproteases [52].

When comparing the nasal mucus proteome of allergic rhinitis patients and healthy controls in pollen season 10 proteins were found to be significantly more abundant in allergic rhinitis. Of these Complement C4-B (C4B), Alpha-1-acid glycoprotein 2 (ORM2) and Phospholipid transfer protein (PLTP) were exclusively present in allergic rhinitis; while Alpha-2-macroglobulin (A2M), Apolipoprotein A-II (APOA2), Vitamin D-binding protein (GC), Complement C3 (C3), Apolipoprotein A-I (APOA1), BPI fold-containing family B member 2 (BPIFB2), and Clusterin (CLU) were also found in healthy controls. Most of these proteins are also found in plasma and may indicate therefore increased plasma exudation in allergic rhinitis [53,54] which may indicate disintegration of the epithelial barrier in allergy [55–57]. C3, A2M, APOA1 and APOA2 were also found to be significantly more abundant in nasal mucus in allergic rhinitis patients in our previous studies [13,14]. Apolipoproteins could act as anti-inflammatory agents apart from their involvement in lipid metabolism [19,20] and their high abundance could be regulated by a local mechanism since plasma levels of APOs did not differ between allergic rhinitis patients and healthy controls and did not correlate to nasal mucus levels [13]. However, further studies are needed to investigate the local production of APOs in the epithelium as a possible alternative mechanism to increased plasma exudation. On the other hand, APOs could also act pro-inflammatory through posttranslational modifications and complexation with other molecules in chronic inflammation [22]. PLTP is responsible for phospholipid transfer between lipoproteins and is elevated in plasma during acute inflammation. Interestingly, it binds to Apolipoprotein A-I, Apolipoprotein E and Clusterin and forms complexes involved in immune response [58]. C3, C4B, ORM2 [59], BPIFB2 [60–62] and GC [63] are pro-inflammatory molecules acting over different ways like the complement system or innate immune cells. As ORM2 was newly identified in the nasal mucus in the present study its role in the upper airway tract needs to be elucidated.

On the basis of the nasal mucus proteome allergic rhinitis patients – contrary to the absence of symptoms out of season – show perennial inflammatory response increasing during pollen season despite reduced defence mechanisms of the immune system and reduced integrity of the epithelial barrier. Moreover, fewer different proteins are expressed in season reflecting a reduced plasticity of the allergic proteome which can also be seen in the lower number of enriched biological processes. In season allergic rhinitis patients lack enrichment of processes like cytoskeleton organization, cell proliferation or cellular homeostasis in contrary to healthy controls. In healthy controls all these important biological processes are enriched in season whereas out of season several of these processes are not found to be enriched. On a genomic level similar findings were seen in primary epithelial cells cultured form healthy controls that were exposed to house dust mite allergen. The mRNA expression profile showed an up-regulation of chemokines, growth factors and structural proteins as well as transcription factors upon provocation whereas epithelial cells from allergics were already in activated state at baseline [64].

5. Conclusion

Contrary to symptom expression allergic rhinitis patients show an increased inflammatory response in their nasal mucus proteome even out of pollen season. In combination with reduced defence mechanisms and an increase in inflammation in season the nasal mucus proteome reflects a decreased plasticity and thus inadequate reaction to allergen stress contrary to healthy controls. Our results also suggest that healthy controls express proteins in their mucus that protect them against harmful pollen content and subsequent sensitization to allergens. Some of our identified proteins could serve as novel biomarkers for allergic rhinitis. Functional analysis of distinct significantly altered proteins will further increase our understanding of the pathophysiology of nasal mucus as defence barrier, which may be the basis of novel therapeutic strategies.

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Conflict of interest and funding

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Transparency document

The Transparency document associated with this article can be found, in the online version.

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